PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

(Chapter II of the Patent Cooperation Treaty)

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference ATHBY/P32968PC	**FOR FURTHER ACTION**	See Form PCT/IPEA/416
International application No. PCT/GB2005/001463	International filing date *(day/month/year)* 15.04.2005	Priority date *(day/month/year)* 15.04.2004
International Patent Classification (IPC) or national classification and IPC INV. C07K16/44 A61K47/48 G01N33/92 G01N33/68		
Applicant ATHERA BIOTECHNOLOGIES AB		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 11 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *sent to the applicant and to the International Bureau)* a total of 2 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) , containing a sequence listing and/or tables related thereto, in electronic form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).

4. This report contains indications relating to the following items:

☒	Box No. I	Basis of the report
☐	Box No. II	Priority
☐	Box No. III	Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
☒	Box No. IV	Lack of unity of invention
☒	Box No. V	Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
☒	Box No. VI	Certain documents cited
☐	Box No. VII	Certain defects in the international application
☒	Box No. VIII	Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28.04.2006	27.07.2006
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized officer Dullaart, A Telephone No. +31 70 340-3290

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
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Box No. I Basis of the report

1. With regard to the **language**, this report is based on

   ☒ the international application in the language in which it was filed

   ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of:
   - ☐ international search (under Rules 12.3(a) and 23.1(b))
   - ☐ publication of the international application (under Rule 12.4(a))
   - ☐ international preliminary examination (under Rules 55.2(a) and/or 55.3(a))

2. With regard to the **elements*** of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report)*:

Description, Pages	
1-30	as originally filed
Claims, Numbers	
1-18	filed with telefax on 28.04.2006
Drawings, Sheets	
1/8-8/8	as originally filed

   ☐ a sequence listing and/or any related table(s) - see Supplemental Box Relating to Sequence Listing

3. ☐ The amendments have resulted in the cancellation of:
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).
   - ☐ the description, pages
   - ☐ the claims, Nos.
   - ☐ the drawings, sheets/figs
   - ☐ the sequence listing *(specify)*:
   - ☐ any table(s) related to sequence listing *(specify)*:

* *If item 4 applies, some or all of these sheets may be marked "superseded."*

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation to restrict or pay additional fees, the applicant has, within the applicable time limit:

   ☐ restricted the claims.

   ☒ paid additional fees.

   ☐ paid additional fees under protest and, where applicable, the protest fee.

   ☐ paid additional fees under protest but the applicable protest fee was not paid.

   ☐ neither restricted the claims nor paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose, according to Rule 68.1, not to invite the applicant to restrict or pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rules 13.1, 13.2 and 13.3 is:

   ☐ complied with.

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☒ all parts.

   ☐ the parts relating to claims Nos. .

Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-18
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-18
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-18
	No:	Claims	

2. Citations and explanations (Rule 70.7):

   see separate sheet

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

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Box No. VI Certain documents cited

1. Certain published documents (Rule 70.10)

   and / or

2. Non-written disclosures (Rule 70.9)

   see separate sheet

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

Reference is made to the following documents:

D1 : **Database Dissertation Abstracts [Online] ProQuest Info&;Learning; 2002 Binder, Christoph Johannes: "Defining innate and adaptive immune mechanisms in the atheroprotective effect of immunization with oxidized low-density lipoproteins"
retrieved from DIALOG accession no. 01907366
Database accession no. AADAA-I3064459**

D2 : **Binder, Christoph J. ET AL: "Pneumococcal vaccination decreases atherosclerotic lesion formation: molecular mimicry between Streptococcus pneumoniae and oxidized LDL"
Nature Medicine, Vol. 9, no. 6, June 2003 (2003-06), pages 736-743, XP002355525 ISSN: 1078-8956**

D3 : **Rose N ET AL: "Autoimmunity: Busting the atherosclerotic plaque"
Nature Medicine, vol. 9, no. 6, 1 June 2003 (2003-06-01), pages 641-642, XP002355526 ISSN: 1078-8956**

D4 : **Binder C J ET AL: "Innate and acquired immunity in atherogenesis"
Nature Medicine, vol. 8, no. 11, 1 November 2002 (2002-11-01), pages 1218-1226, XP002355527 ISSN: 1078-8956**

D5 : **Shaw P X ET AL: "The autoreactivity of anti-phosphorylcholine antibodies for atherosclerosis-associated neo-antigens and apoptotic cells"
JOURNAL OF IMMUNOLOGY 15 JUN 2003 UNITED STATES, vol. 170, no. 12, 15 June 2003 (2003-06-15), pages 6151-6157, XP002355528 ISSN: 0022-1767**

D6 : **Binder Christoph J ET AL: "Molecular mimicry between epitopes of oxidized LDL and Streptococcus pneumoniae"
ABSTRACTS FROM AMERICAN HEART ASSOCIATION SCIENTIFIC SESSIONS 2000, [Online] 12 November 2000 (2000-11-12), XP002355529 NEW ORLEANS, LOUISIANA, US, Abstract ID: 108867 Retrieved from the Internet: URL:http://aha.agora.com/abstractviewer>; [retrieved on 2005-11-10]**

D7 : **Purkall D ET AL: "Opsonization of Actinobacillus actinomycetemcomitans by immunoglobulin G antibody reactive with phosphorylcholine"
Infection and Immunity, vol. 70, no. 11, 2002, pages 6485-6488, XP002355530 ISSN: 0019-9567**

D8 : **WO 99/33522 A (BOARD OF REGENTS, THE UNIVERSITY OF TEXAS SYSTEM;**

SCHROIT, ALAN, J) 8 July 1999 (1999-07-08)

D9 : US 5 455 032 A (KENNY ET AL) 3 October 1995 (1995-10-03)

D10 : Shoji Tetsuo ET AL: "Inverse relationship between circulating oxidized low density lipoprotein (oxLDL) and anti-oxLDL antibody levels in healthy subjects"
Atherosclerosis, Vol. 148, no. 1, January 2000 (2000-01), pages 171-177, XP002355531 ISSN: 0021-9150

D11 : WO 01/32070 A (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA; WITZTUM, JOSEPH; TSIMIKAS) 10 May 2001 (2001-05-10)

D12 : WO 02/080954 A (FORSKARPATENT I SYD) 17 October 2002 (2002-10-17)

D13 : WO 01/68119 A (KAROLINSKA INNOVATIONS AB; HANSSON, GOERAN, K; STEMME, STEN; NICOLETTI) 20 September 2001 (2001-09-20)

D14 : WO 90/12632 A (THE UNITED STATES OF AMERICA, REPRESENTED BY THE S) 1 November 1990 (1990-11-01)

D15 : KOH-ZOH KAMEYAMA ET AL: "CONVENIENT PLASMID VECTORS FOR CONSTRUCTION OF CHIMERIC MOUSE/HUMAN ANTIBODIES"
FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, Vol. 244, no. 2, 27 February 1989 (1989-02-27), pages 301-306, XP000007812 ISSN: 0014-5793

D16 : EP 0 466 505 A (FUJITA HEALTH UNIVERSITY; TAKARA SHUZO CO. LTD) 15 January 1992 (1992-01-15)

D17 : WO 94/14454 A (ENTREMED, INC) 7 July 1994 (1994-07-07)

D18 : US 5 955 584 A (DITLOW ET AL) 21 September 1999 (1999-09-21)

D19 : KEARNEY JOHN F: "Immune recognition of OxLDL in atherosclerosis"
JOURNAL OF CLINICAL INVESTIGATION, Vol. 105, no. 12, June 2000 (2000-06), pages 1683-1685, XP002367018 ISSN: 0021-9738

D20 : CHYU KUANG-YUH et al: "Changes in innate and adaptive humoral immune responses and indices of atherosclerosis in aging."
Journal of the American College of Cardiology, vol. 43, no. 5, Supplement A, 3 March 2004 (2004-03-03), page 499A, abstract no. 1122-173, XP002367019
& 53rd Annual Scientific Session of the American College of Cardiology; New Orleans, LA, USA; March 07-10, 2004 ISSN: 0735-1097

D21 : WO 93/18161 A (THE ROCKEFELLER UNIVERSITY) 16 September 1993 (1993-09-16)

D22 : US 5 475 100 A (HASHINO ET AL) 12 December 1995 (1995-12-12)

D23 : SHAW PETER X ET AL: "Natural antibodies with the T15 idiotype may act in atherosclerosis, apoptotic clearance, and protective immunity" JOURNAL OF CLINICAL INVESTIGATION, Vol. 105, no. 12, June 2000 (2000-06), pages 1731-1740, XP002204419 ISSN: 0021-9738

Re Item IV.

The separate inventions/groups of inventions are:

No.	Claims	
1.	1-8	Use of an antibody specific for a phosphorylcholine conjugate in the treatment of atherosclerosis or related disease, and corresponding method of prophylactic or therapeutic treatment.
2.	9-18	Use of a phosphorylcholine conjugate for assessing a patient's risk of developing or progression of ischemic cardiovascular disease as defined in these claims.

They are not so linked as to form a single general inventive concept (Rule 13.1 PCT) for the following reasons:

The two problems underlying the present application are to provide a therapeutic or prophylactic use or method for atherosclerosis (claims 1-8), and a use for assessing a patient's risk of developing or progression of ischemic cardiovascular disease (claims 9-18). As solution to the first problem, an anti-PC antibody is proposed. To the second of these problems, an immunogenic conjugate of phosphorylcholine (PC) is proposed.
The common technical feature linking these different subjects is the relationship between anti-PC immune response or anti-PC antibodies and the reduction of atherosclerosis risk. This link has, however, already been described in the prior art.
More specifically, document D10 mentions on page 176, at the beginning of the left hand column that "*patients with a history of myocardial infarction had lower titer of IgM-class oxLDL Ab than those without. In addition, the present study has revealed the inverse relationship between oxLDL Ab titer and plasma oxLDL concentration in the healthy*

human subject".

This documents thus anticipates the technical feature linking the different subjects contained in the present application. Therefore, this technical feature can no longer serve as special technical feature in the sense of Rule 13 PCT, linking the different subjects together.
Since there is no other technical feature, that could fulfil the role of special technical feature in the sense of Rule 13 PCT, the present application lacks unity of invention, containing the subject-matters as listed.
In principle, each of the compounds mentioned in the claims represents a different invention. However, in order to reduce the number of subjects as much as possible, the compounds have been regrouped according to structural similarities, and to the different problems to be solved.
As the applicant has paid both a search fee and an examination fee for all inventions, both inventions can be examined.

Re Item V.

2 Invention 1

Document D1 discloses that anti-PC antibody T15 = EO6 protects against S. Pneumoniae and inhibits atherogenesis. The antibody is elicited by means of vaccination.
Document D2 discloses the anti-atherogenic effect of pneumococcal immunisation. The underlying mechanism is the fact, that in both cases the antibody is specific for phosphorylcholine.
Document D3 discloses that, "contrary to the more well-accepted notion that autoimmunity associated with atherosclerosis leads to disease, Binder, Hörkkö et al.3, in this issue, propose that autoimmunity can be protective. The authors provide evidence that a natural autoantibody to oxidized LDL (oxLDL), called T15, does not produce atherosclerosis in a mouse model, but rather decreases the extent of the disease. The data suggest that vaccines that boost T15 levels might protect against atherosclerosis".
Document D4 mentions that "an increased titer of EO6 antibodies would be expected to be protective, as these antibodies potently block macrophage uptake of oxLDL".
Document D5 discloses that the anti-PC antibody also reacts with antigens linked to

atherosclerosis.
Document D6 suggests the link between vaccination and the reduction of atherogenesis.
Document D7 discloses the antimicrobial effect of anti-PC antibody.
Document D8 discloses the conjugates of PC with different proteins, which elicit an anti-PC antibody response in vivo.
Document D9 discloses the conjugates of PC with different proteins, which elicit an anti-PC antibody response in vivo. The detection of these antibodies is given the last example, with the results in table 2.

Documents D1 to D 6 each suggest that vaccines which increase antibodies like EO6 protect against atherosclerosis.
Documents D7 to D9 describe, that conjugation of phosphorylcholine to a large peptide like BSA elicits such an immune response.
Document D11 discloses antibody IK17. This antibody detects OxLDL; a marker for atherosclerosis. Hence it is proposed for targeting atherosclerotic drugs.
Also, both documents D12 and D13 disclose the use of a different antigen to elicit anti-atherosclerotic immune response.
Document D15 discloses the use of a hybridoma for producing an anti-phosphorylcholine antibody. This antibody has retained its specificity for the PC-OVA conjugate.
Document D17 discloses a sterol-based vaccine against atherosclerosis.
Perhaps more specifically, document D16 discloses the production of antibodies specific for PC-KLH, as demonstrated by example 4.

Document D10 discloses the inverse relationship between circulating oxidized low density lipoprotein (OxLDL) and anti-OxLDL antibody levels in healthy subjects. Invention 1 of the present application can be distinguished from this prior art by the fact, that these findings are applied in the therapeutic treatment of atherosclerosis, by using such an antibody.

The closest prior art is found in any of documents D1 to D6, which each solve the same problem of treating atherosclerosis. The presently claimed use according to invention 1 can be distinguished from this prior art by the fact, that instead of treating atherosclerosis using a vaccine, the disease is treated using an antibody.
This antibody is known from documents D7 to D9, D11 to D13 and D15 to D17. However, in none of these documents, the intended use of the antibody is therapeutic. Also, in most

of these documents, the antibody is also elicited using a PC conjugate. Therefore, the skilled person would not have found the suggestion to use an antibody against PC in the treatment of atherosclerosis. Rather, these documents confirm that the use of a vaccine is efficient, and therefore probably a better way of treating atherosclerosis.
Therefore, invention 1 appears to meet the requirements of Article 33.3 PCT for inventive step.

Invention 2

Document D19 discloses an increase in anti-phosphorylcholine antibodies due to atherosclerosis.
Document D20 discloses an increase in anti-phosphorylcholine IgM and IgG antibodies due to atherosclerosis.
These documents each clearly determine the increase of anti-phosphorylcholine antibodies in atherosclerosis. These document do not explicitly mention the link with ischemic cardiovascular diseases.
Document D21 discloses the detection of cells expressing anti-phosphorylcholine antibody by reaction with a PC-albumin conjugate.
Document D23 discloses the role of anti-PC antibodies in atherogenesis.
These documents each clearly determine the increase of anti-phosphorylcholine antibodies. These document do not explicitly mention the link with ischemic cardiovascular diseases.
Atherosclerosis is a risk factor in cardiovascular diseases well known to the skilled person. However, the presently claimed use proposes to detect the risk of cardiovascular disease in the opposite way, i.e., by linking a lower blood level of anti-PC antibodies to an increased risk. As this use according to the presently claimed invention 2 is contradicted by the prior art, the skilled person would have been taught away from this invention. In view of these reasons, the presently claimed invention 2 fulfills the requirements of inventive step in the sense of Article 33.3 PCT.

Re Item VI
Certain documents cited

Certain published documents

Application No Patent No	Publication date *(day/month/year)*	Filing date *(day/month/year)*	Priority date *(valid claim)* *(day/month/year)*
US 2004/0185039	23-9-2004	29-8-2003	30-8-2002

Re Item VIII
Certain observations on the international application

In present claims 9-18, the phosphorylcholine conjugate is only partially defined. Since this conjugate is the very basis of the presently claimed inventions, these claims do not meet the requirements of Article 6 PCT in that the matter for which protection is sought is not clearly defined.
Moreover, nowhere in the present application, latex beads to which phosphorylcholine is conjugated, are prepared. Therefore, claims 7 and 17 do not meet the requirements of Article 5 PCT for sufficiency of disclosure.

CLAIMS

1. Use of a pharmaceutical composition comprising ~~at least one phosphorylcholine conjugate, or~~ an antibody preparation, for example a monoclonal antibody, with specificity to a phosphorylcholine conjugate, in the manufacture of a medicament for immunization and treatment of ~~mammals, including~~ humans, against atherosclerosis or an atherosclerotic related disease.

2. A method for immunization and treatment of a ~~mammal, including a~~ human, against atherosclerosis or an atherosclerotic related disease, the method comprising the step of administering to the ~~mammal~~ human a pharmaceutical composition comprising ~~at least one phosphorylcholine conjugate, or~~ an antibody preparation, for example a monoclonal antibody, with specificity to a phosphorylcholine conjugate

3. The use of claim 1 or method of claim 2 wherein the medicament is for administration by injection or wherein the composition is administered by injection.

4. The use or method of any one of the preceding claims wherein the phosphorylcholine is linked to a carrier via a spacer.

5. The use or method according to claim 4, wherein the carrier is a protein.

6. The use or method according to claim 5, wherein the protein is KLH (keyhole limpet hemocyanin) or human serum albumin (HSA).

7. The use or method according to claim 4 wherein the carrier is latex beads.

~~8. The use of one or more of the phosphorylcholine conjugates as defined in~~ any one of the preceding claims in the manufacture of a pharmaceutical composition, optionally in combination with an adjuvant, for ~~immunotherapy or therapy for the treatment of ischemic cardiovascular diseases.~~

8 9. A method of prophylactic or therapeutic treatment of a ~~mammal, for example a~~ human being, suffering from atherosclerosis or facing the risk of developing ischemic cardiovascular disease, whereby a therapeutically effective amount of ~~at least one phosphorylcholine conjugate or~~ an

antibody preparation, for example a monoclonal antibody, with specificity to a phosphorylcholine conjugate is administered..

~~10. Method of diagnosing the presence or absence of IgM or IgG antibodies related to increased or decreased risk of developing ischemic cardiovascular diseases, using a phosphorylcholine conjugate.~~

14. ~~11. Method~~ Use according to any one of claim 9 to 13 wherein phosphorylcholine is linked to a carrier via a spacer.

15. ~~12. Method~~ Use according to claim 14 wherein the carrier is a protein

16. ~~13. Method~~ Use according to claim 15 wherein the protein is KLH (keyhole limpet hemocyanin) or human serum albumin (HSA).

17. ~~14. Method~~ Use according to claim 14, wherein the carrier is latex beads.

18. ~~15. Method~~ Use according to any one of claims 9 to 17, wherein the assay is an immunoassay.

9. ~~16.~~ Use of a phosphorylcholine conjugate in a method for assessing a human patient's risk of developing or progression of ~~ischemic~~ cardiovascular disease in which the patient's levels of ~~IgM or IgG~~ antibodies reactive with the phosphorylcholine conjugate are assessed, wherein low levels of antibody reactive with the phosphorylcholine conjugate are predictive of the occurrence of cardiovascular disease in a healthy human patient.

10. The use of Claim 9 wherein the cardiovascular disease is ischemic cardiovascular disease.

11. The use of Claim 9 wherein the cardiovascular disease is atherosclerosis.

12. The use of any one of Claims 9 to 11 wherein the patient's levels of IgM antibodies reactive with the phosphorylcholine conjugate are assessed.

13. The use of any one of Claims 9 to 11 wherein the patient's levels of IgG antibodies reactive with the phosphorylcholine conjugate are assessed.